

Animal Model

Stable β -Secretase Activity and Presynaptic Cholinergic Markers During Progressive Central Nervous System Amyloidogenesis in Tg2576 Mice

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We examined presynaptic cholinergic markers and β -secretase activity during progressive central nervous system amyloidogenesis in Tg2576 Alzheimer mice (transgenic for human amyloid precursor protein Swedish mutation; hAPP^{Swe}). At 14, 18, and 23 months of age there were no significant differences between wild-type and transgenic mice in four distinct central nervous system cholinergic indices—choline acetyltransferase and acetylcholinesterase activities, and binding to vesicular acetylcholine transporter and Na⁺-dependent high-affinity choline uptake sites. A novel enzyme-linked immunosorbent assay measuring only the secreted human β -secretase cleavage product (APPs β swe) of APP^{Swe} also revealed no change with aging in Tg2576 mouse brain. In contrast, transgenic but not wild-type mice exhibited an age-dependent increase in soluble A β ₄₀ and A β ₄₂ levels and progressive amyloid deposition in brain. Thus, aging Tg2576 mice exhibited presynaptic cholinergic integrity despite progressively increased soluble A β ₄₀ and A β ₄₂ levels and amyloid plaque density in brain. Older Tg2576 mice may best resemble preclinical or early stages of human Alzheimer's disease with preserved presynaptic cholinergic innervation. Homeostatic APPs β swe levels with aging suggest

that progressive amyloid deposition in brain results not from increased β -secretase cleavage of APP but from impaired A β /amyloid clearance mechanisms. (Am J Pathol 2002, 160:731–738)

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive dementia and the accumulation of neuritic plaques and neurofibrillary tangles in limbic and neocortical brain regions. Cholinergic deficits were the first neurochemical changes recognized in AD brain^{1–3} as shown by decreases in choline acetyltransferase (ChAT; acetyl-CoA: choline O-acetyltransferase; EC 2.3.1.6) and acetylcholinesterase (AChE; acetylcholine acetylhydrolase; EC 3.1.1.7) activities with perhaps compensatory increases in Na⁺-dependent high-affinity choline uptake (SDHACU) sites.^{4–6} The major cholinergic nuclei of brain affected are in the basal forebrain^{7,8} that project to the neocortex, entorhinal area, and hippocampus.^{9,10} To date, AChE inhibitors are the only class of drugs approved by the United States Food and Drug Administration for the treatment of cognitive deficits caused by AD.

Because basal forebrain cholinergic neurons project widely to hippocampus and neocortex, it was suggested that cholinergic denervation is linked to or even induces neurodegenerative changes, including amyloid deposition, in AD cortex.^{11–13} This cholinergic hypothesis has been supplanted by recent genetic, biochemical, and pathological evidence supporting the amyloid hypothesis of AD pathogenesis.¹⁴ Despite recent progress, however, the temporal course of cholinergic deficits in the

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clinical and pathological staging of human AD remains controversial. Some studies suggest that cortical cholinergic deficits occur early and in parallel with dementia and amyloid plaque deposition.^{15,16} Other studies of human AD brain demonstrate cholinergic deficits only in very late stages of disease.^{17,18}

Studies of central nervous system (CNS) cholinergic markers in human amyloid precursor protein transgenic (hAPP tg) mouse models of AD are limited, and report only on immunohistochemical and microscopic analysis. For example, brain sections from hAPP Swedish mutation (hAPPswe) tg mice (not Tg2576) at 12 months of age exhibit AChE-immunoreactive dystrophic fibers in the vicinity of amyloid plaques.¹⁹ Similarly, in 17- to 22-month hAPP V642I (London mutation) tg mice AChE-positive fibers are reorganized within the hippocampus and amyloid plaques are surrounded by dystrophic AChE-positive fibers.²⁰ In 8-month-old hAPPswe (Tg2576)/hPresenilin-1 M146L double-tg mice vesicular acetylcholine transporter (VAcHT)-immunoreactive synapses are decreased in frontal cortex and the size of these synapses is reduced in hippocampus; at this age there is extensive amyloid plaque deposition in brain. In contrast, in Tg2576 mice at 8 months of age there are no amyloid plaques in brain and VAcHT-immunostained synapses are increased in frontal and parietal cortex.²¹

Based on this evidence, we pursued a macroscopic study of aging Tg2576 mouse brain by measuring four cholinergic markers—VAcHT, SDHACU, ChAT, and AChE—during progressive CNS amyloidogenesis in 14-, 18-, and 23-month-old mice. With aging, these mice exhibit a partial AD-like phenotype that includes learning and memory deficits and pathological findings of amyloid plaque deposition, neuritic changes, gliosis, inflammatory responses, phosphorylated tau epitopes, α -synuclein-immunopositive (Lewy-like) neurites, and increased A β 40 and A β 42 levels, but not neuronal loss or neurofibrillary tangles.^{22–40} VAcHT sites are a specific marker of cholinergic terminals.^{41–44} When labeled vesamicol is used with selective blockers, VAcHT may be quantified and is useful for assessing loss of cholinergic projections in AD brain both *in vitro* and *in vivo*.^{45–47} During AD progression *in vivo* VAcHT binding is less affected than postmortem ChAT activity measurements would suggest.⁴⁵ ChAT is the hallmark of cholinergic fibers and is present in the striatum/cerebral cortex/cerebellum of adult rats in the ratios ~26:9:1.⁴⁸ Hemicholinium-3 (HC-3) binds specifically to SDHACU sites present on cholinergic neurons;⁴⁹ choline uptake is the rate-limiting step in acetylcholine biosynthesis.

In addition to A β 40 and A β 42 enzyme-linked immunosorbent assay (ELISA) and histochemical analysis of brain sections, we used a novel ELISA that detects only the secreted β -secretase (BACE) cleavage fragment (APPs β swe) of human APPswe.⁵⁰ By measuring both secreted products of BACE cleavage of APPswe (A β peptides and APPs β swe) in Tg2576 mouse brain we may indirectly assess a hypothesized increase in BACE activity with aging.

Materials and Methods

Animals

Three male Tg2576 mice (hAPPswe in a C57B6/SJL genetic background;²² Mayo Clinical Ventures, Minneapolis, MN) were crossed with (C57B6 \times SJL) F₁ females (Jackson Laboratories, Bar Harbor, ME). At one month of age, progeny were screened for hAPPswe transgenesis by polymerase chain reaction of genomic DNA extracted from tails. The hAPP primers used were 5'-GTG GAT AAC CCC TCC CCC AGC CTA GAC CA-3' and 5'-CTG ACC ACT CGA CCA GGT TCT GGG T-3'. In transgene-positive mice a 450-bp polymerase chain reaction product was detected on 4% agarose gels. All tg mice were hAPPswe hemizygous (homozygous is lethal). Nontransgenic littermates were used as wild-type (wt) controls. Mice were housed under specific pathogen-free conditions with a 12-hour light-dark cycle and food and water was provided *ad libitum*. Animal care was in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health. Housing facilities were accredited by the American Association for the Accreditation of Laboratory Animal Care. All experimental procedures were approved by the University of Michigan Committee on Use and Care of Animals.

Frozen and Paraformaldehyde-Fixed Brain

Six wt and six tg mice of three age groups (14, 18, and 23 months) were used in this study. All wt mice were male except for one female in the 23-month age group; all tg mice were female except for one male in the 18-month age group. Male tg mice are more aggressive than wt mice and must be singly-housed; thus, housing costs for aging tg males are prohibitive. Mice were sacrificed painlessly and removed brains were divided sagittally. The left half was frozen onto a gauze-covered glass microscope slide on dry ice with M-1 embedding matrix (Shandon Lipshaw, Pittsburgh, PA). The right half was fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.4, at 4°C and processed for paraffin embedding. Sagittal sections of 20 μ m were obtained from frozen hemi-brains for as long as striatum, hippocampus, and cerebellum were visible. Every five consecutive sections were grouped and numbered from 1 to 20. The sections from odd numbered groups were collected onto poly-L-lysine-treated glass slides and air-dried at room temperature for 8 hours to overnight. Even numbered sections were microdissected to obtain four brain regions: cortex, striatum, hippocampus, and cerebellum. Both microdissected tissues and cryostat sections were stored at -70°C until further analysis. Paraformaldehyde-fixed hemi-brains were sectioned coronally at 5- to 8- μ m thickness and sections were processed for hematoxylin and eosin, Bielschowsky silver, and Congo Red staining.

[³H]Vesamicol Binding to VAcHT Sites

Tissue sections mounted onto glass slides were thawed at room temperature and prewashed for 5 minutes in

PBS-ethylenediaminetetraacetic acid (EDTA), pH 7.4, at room temperature. Sections were then incubated with [³H]vesamicol (specific activity, 35.0 Ci/mmol; final concentration, 10 nmol/L; New England Nuclear, Boston, MA) in PBS-EDTA, pH 7.4, at room temperature for 60 minutes with intermittent agitation. To obtain nonspecific binding, sections were treated as above with the addition of 200 nmol/L (–)methylaminobenzovesamicol. Tissue sections were rinsed in PBS-EDTA, pH 7.4, at 4°C for 1 minute twice, washed in distilled water at 4°C for 5 seconds, air-dried overnight, and exposed to Hyperfilm (Amersham, Arlington Heights, IL) for 6 weeks.

[³H]Hemicholinium-3 (HC-3) Binding to SDHACU Sites

The [³H]HC-3 binding assay was performed as above except: 1) the final concentration of [³H]HC-3 (specific activity, 137.2 Ci/mmol; New England Nuclear) was 2 nmol/L, 2) nonspecific binding was performed with the addition of unlabeled 10 μmol/L HC-3, and 3) the incubation time was 2 hours instead of 1 hour.

Quantitation of autoradiograms from binding assays was performed with the MCID-M5+ image analysis system (Imaging Research Inc., St. Catharines, Ontario, Canada). Briefly, the image was captured by a charge-coupled device video camera and displayed on a computer screen. The total and nonspecific binding activities were measured by outlining the cortex, striatum, hippocampus, and cerebellum. Specific binding was calculated by subtracting nonspecific from total binding. Measures to minimize sampling errors and variability of autoradiography included quantitative image analysis of 10 (for HC-3 binding) or 20 (for vesamicol binding) consecutive tissue sections for each mouse hemi-brain, and by using a standardized [³H] calibration panel with each autoradiogram.

ChAT Activity

ChAT activity was measured by the radioisotopic method of Fonnum.⁵¹ Briefly, 60 μl of 500 nmol/L NaCl, 83 nmol/L PBS (pH 7.4), 13 mmol/L choline bromide, 33 mmol/L EDTA, 0.17 mmol/L physostigmine, and 0.083% Triton X-100, was added to glass tubes at 4°C. Microdissected cortex, hippocampus, and cerebellum were homogenized in 0.5 ml, and striatum in 1 ml, of 100 mmol/L of PBS, pH 7.4, at 4°C with intermittent sonication. Twenty μl of samples and 20 μl of [¹⁴C]acetyl CoA (2.25 μCi/ml; final concentration, 0.2 mmol/L; New England Nuclear) were added to the tubes at 4°C. The reaction time was 15 minutes in a shaking water bath at 37°C. The reaction cocktail was washed twice with 2.5 ml of 10 mmol/L PBS, pH 7.4, at 4°C and collected in a scintillation vial. Two ml of acetonitrile/tetraphenylboron and 10 ml of toluene scintillant were added to the vial with repeated vigorous agitation. The samples were left overnight and counted in a scintillation counter. All ChAT assays were measured in triplicate. Protein was determined by a modified bicin-

choninic acid method,⁵² all samples were measured in triplicate.

Acetylcholinesterase (AChE) Activity

The colorimetric assay for acetylcholinesterase activity by Ellman and colleagues⁵³ was used. A microtiter plate (Nunc 96-well VWR Scientific, West Chester, PA) was placed on ice and 300 μl of 0.1 mol/L phosphate buffer, pH 8.0, was added, followed by 2 μl of 0.075 mol/L acetylthiocholine iodide and 10 μl of 0.01 mol/L dithio-bisnitrobenzoic acid. The brain region homogenates were added as 5-μl aliquots into triplicate wells. Also included were a nonenzymatic protein (bovine serum albumin) and no enzyme blank. The microtiter plate was removed from the ice and gently shaken at room temperature until the plate was read at 405 nm with a Beckman Biomek 1000. The resultant absorbance values were converted into enzyme activity (pmol/min/μg protein).

Aβ40, Aβ42, and APPsβswe ELISA

Brain homogenates for ELISA were the same samples used for measuring ChAT activity. Homogenates were centrifuged at 7840 × g for 5 minutes at 4°C to remove insoluble material. A sandwich ELISA was performed on the supernatants as described^{54,55} using BAN50 as the capture antibody and either horseradish peroxidase-coupled BA-27 or BC-05 as the detection antibody for Aβ40 or Aβ42, respectively. BAN-50 is a monoclonal antibody specific for Aβ1-10. All samples were measured in duplicate. APPsβswe was measured with a novel ELISA specific to the β-secretase cleaved fragment of human APPswe.⁵⁰

Statistical Analysis

A repeated mixed model of three fixed factors (age, genotype, and region) was used to evaluate factor effects in ChAT and AChE activities, HC-3 binding, and vesamicol binding. Repeated mixed models of two fixed factors (age and region) were used to evaluate factor effects in Aβ40, Aβ42, and APPsβswe levels and the Aβ42/Aβ40 ratio in tg mice. We assumed compound symmetry for the covariance structure in the models. A significance level of 5% was set for each statistical test. Pairwise comparisons between age groups or brain regions were used if applicable. The notation A ~ B ~ C > D means that 1) the averages A, B, C, and D are arranged in decreasing order; 2) D is significantly different from the others (A, B, and C); and 3) no significant difference exists between A, B, and C. No adjustment of the significance level was made for multiple tests. GLM and MIXED procedures in the statistical software SAS (PC version 8.1; SAS Institute Inc., Cary, NC) were used for the analyses.

Table 1. Pathologic Findings in hAPPswe tg Mouse Brains Based on Bielschowsky Silver and Congo Red Staining of Brain Sections

	Age (months)		
	14	18	23
Amyloid plaques			
Cerebral cortex	+	++	+++
Hippocampus	-	+/-	++
Striatum	-	-	+
Thalamus	-	-	+
Cerebellum	-	-	-
Vascular amyloid	+/-	+/-	+

-, None; +/-, rare; +, occasional; ++, frequent; and +++, numerous plaques. Vascular amyloid was both meningeal and parenchymal.

Results

Amyloidogenesis Increases with Age in Tg2576 Mouse Brain

The spatial and temporal progression of amyloid plaque deposition in Tg2576 mouse brain was cortex \gg hippocampus > striatum = thalamus, but no plaques were found in cerebellum even at 23 months of age (Table 1). Although amyloid plaques were rare in the cortex of the 14-month-old age group and not seen in other brain regions, there were significant levels of soluble A β 40 and A β 42 (see Figure 5), suggesting that elevations in A β 40 and A β 42 concentrations precede their deposition as amyloid. The levels of A β 40 and A β 42 increased progressively with age and in parallel in all brain regions from tg mice. At 14 months there were no significant differences in A β 40 or A β 42 levels between the four brain regions. At 18 and 23 months, the relative levels of soluble CNS A β 40 were cortex > striatum ~ hippocampus > cerebellum; for A β 42, relative levels were cortex ~ hippocampus ~ striatum > cerebellum at 18 and 23 months, with cortex > hippocampus only at 23 months. Interestingly, although the striatum and hippocampus had similar levels of soluble A β 40 and A β 42, amyloid plaques were more abundant in the hippocampus. A β 42 levels were consistently greater than A β 40 at all ages, and the A β 42/A β 40 ratio significantly peaked at 18 months and declined at 23 months ($P < 0.03$; see Figure 5) suggesting

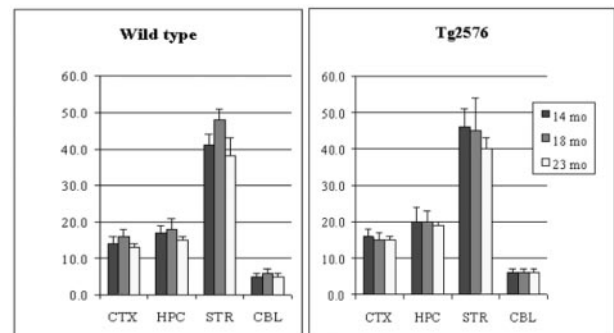


Figure 1. Specific vesamicol (VAcHT) binding in cortex (CTX), hippocampus (HPC), striatum (STR), and cerebellum (CBL) of aging Tg2576 mouse brain. Data shown are mean \pm SD of dpm/ μ g protein, $n = 4$.

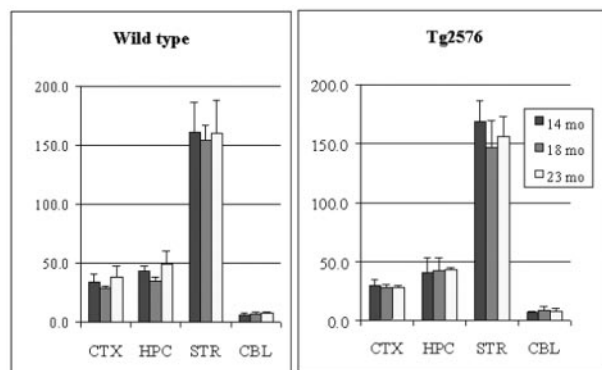


Figure 2. Hemicholinium-3 binding (SDHACU) in cortex (CTX), hippocampus (HPC), striatum (STR), and cerebellum (CBL) of aging Tg2576 mouse brain. Data shown are mean \pm SD of dpm/ μ g protein, $n = 3$. Similar results were obtained with a duplicate analysis, $n = 3$.

that A β 42 precedes A β 40 deposition in brain. No amyloid plaques were detected in 14-, 18-, and 23-month-old wt mouse brain (data not shown). As expected for the human-specific ELISA, no soluble A β 40 and A β 42 was detected in wt mouse brain (data not shown).

Cholinergic Markers Are Preserved in Aging Wt and Tg2576 Mouse Brain

In both wt and tg mouse brain, the order of vesamicol binding was striatum > hippocampus > cortex > cerebellum ($P < 0.0001$, Figure 1), whereas the order of HC-3 binding, ChAT activity, and AChE activity was striatum > hippocampus ~ cortex > cerebellum ($P < 0.001$, Figures 2, 3, and 4). In hAPPswe mice, the 14-month age group had a higher level of ChAT activity in striatum than the 18- and 23-month age groups, but this difference was not statistically significant (Figure 3). In fact, there were no significant differences between wt and tg mice for four cholinergic markers in three age groups and four brain regions. Thus, in the Tg2576 line neither hAPPswe transgenesis nor progressive amyloidogenesis with aging alter these cholinergic markers. Our results for four distinct cholinergic markers were consistent with each other and with the literature.^{48,56,57}

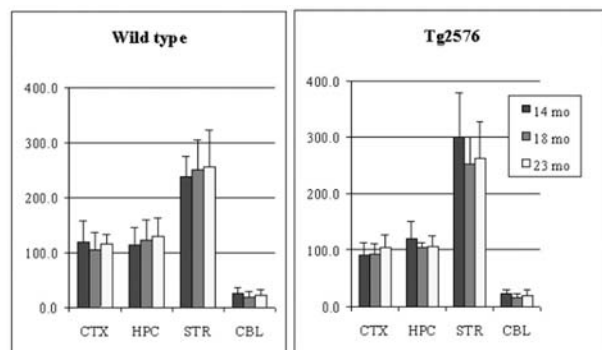


Figure 3. ChAT activity in cortex (CTX), hippocampus (HPC), striatum (STR), and cerebellum (CBL) of aging Tg2576 mouse brain. Data shown are mean \pm SD of nmol/h/mg protein, $n = 6$.

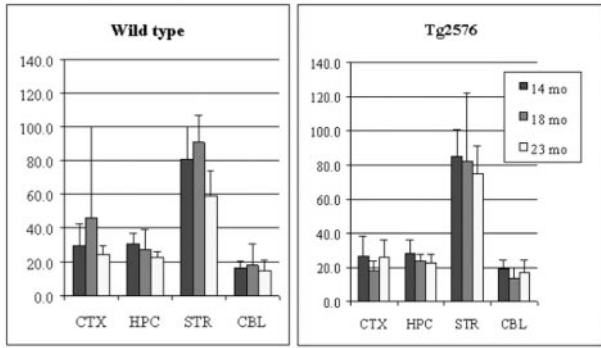


Figure 4. Acetylcholinesterase (AChE) activity in cortex (CTX), hippocampus (HPC), striatum (STR), and cerebellum (CBL) of aging Tg2576 mouse brain. Data shown are mean \pm SD of pmol/min/ μ g protein, $n = 6$.

The Secreted β -Secretase Product of hAPPswe Is Static in Aging Tg2576 Mouse Brain

By the use of a novel, sensitive, and specific ELISA for APPs β swe,⁵⁰ we may indirectly assess a hypothesized increase in BACE activity with aging. However, APPs β swe levels were static in all brain regions as follows: cortex \sim hippocampus \sim striatum $>$ cerebellum (Figure 6). In contrast, in the same samples $A\beta$ 40 and $A\beta$ 42 levels progressively increased with aging (Figure 5). As expected for the human-specific ELISA, no APPs β swe was detected in wt mouse brain (data not shown). This ELISA detects neither mouse APP and its secreted α - β -secretase fragments nor full-length human APPswe and its α -secretase fragment.⁵⁰

Discussion

The presynaptic cholinergic markers VAcHT binding, SDHACU binding, and ChAT activity, as well as AChE activity, were preserved in Tg2576 mouse brain at 14, 18, and 23 months of age. In contrast, progressive CNS amyloidogenesis was demonstrated by increasing soluble $A\beta$ 40 and $A\beta$ 42 levels and by histochemical staining of sections. Because behavioral deficits precede amyloid deposition in brain, soluble $A\beta$ peptides and oligomers may have synaptotoxic and neurotoxic effects independent of plaque formation.^{58,59} In 14- to 23-month-old mice, however, the neurochemical basis for learning and memory deficits does not seem to be because of cholinergic denervation. Rather, glutamate receptor dysregulation has been implicated.³⁵

Our results extend published data on Tg2576 mice^{22,36} by examination of $A\beta$ 40 and $A\beta$ 42 levels in brain regions instead of whole brain. Hsiao and colleagues²² report higher levels of $A\beta$ 40 compared to $A\beta$ 42 in brains of tg mice only up to 12 months of age, but with aging the $A\beta$ 42 level increased 14-fold compared to fivefold for $A\beta$ 40. In contrast to Kawarabashi and colleagues,³⁶ we found that $A\beta$ 42 levels exceeded $A\beta$ 40 levels in old Tg2576 mouse brain. This discrepancy may reflect a difference in extraction methods. Similar to our mouse data, in human brain levels of the more fibrillogenic $A\beta$ 42 are consistently greater than $A\beta$ 40 in cortex

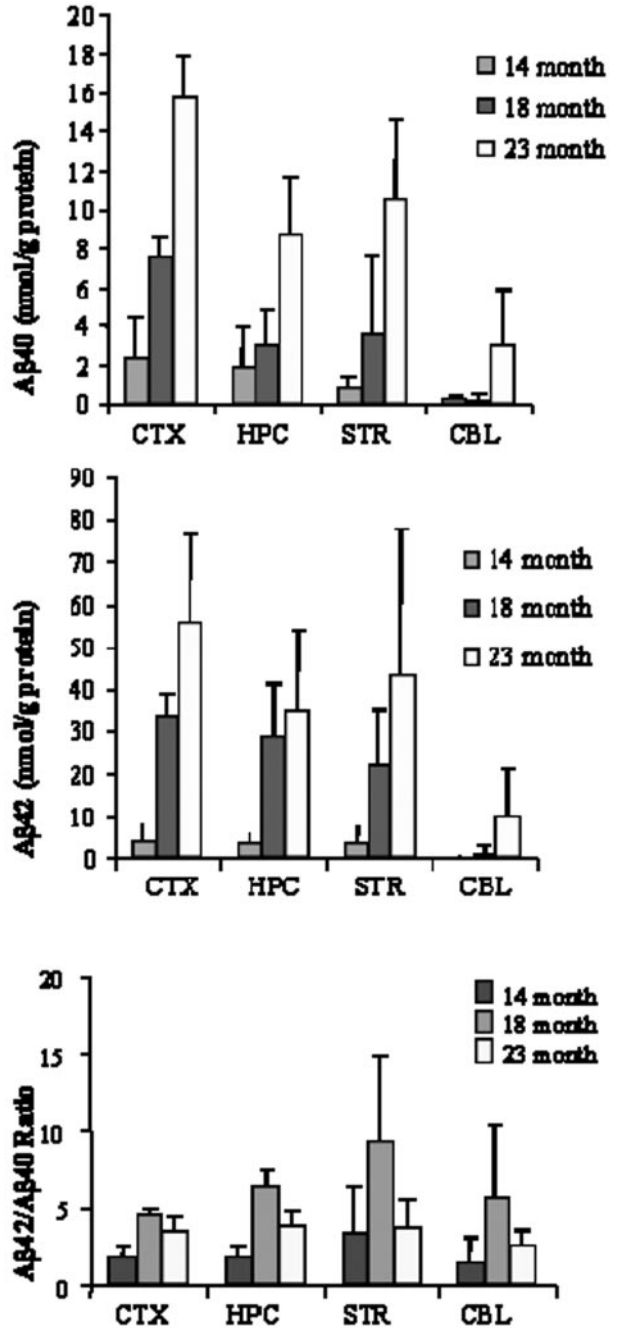


Figure 5. Soluble $A\beta$ 40 and $A\beta$ 42 levels and the $A\beta$ 42/ $A\beta$ 40 ratio in cortex (CTX), hippocampus (HPC), striatum (STR), and cerebellum (CBL) of aging Tg2576 mouse brain. Data shown are mean \pm SD, $n = 3$.

from nondemented patients and through all stages of AD.^{60,61} The $A\beta$ 42/ $A\beta$ 40 ratio increased at 18 months (compared to 14 months) and then declined at 23 months of age. This pattern was observed in all brain regions, and suggests that $A\beta$ 42 preferentially accumulates earlier than $A\beta$ 40 in aging Tg2576 mouse brain. Finally, homeostatic soluble APPs β swe levels suggest that $A\beta$ accumulates with aging not because of increased BACE activity but because of impaired $A\beta$ /amyloid clearance mechanisms in aging brain. This finding is in agreement with static BACE expression in aging Tg2576 mouse

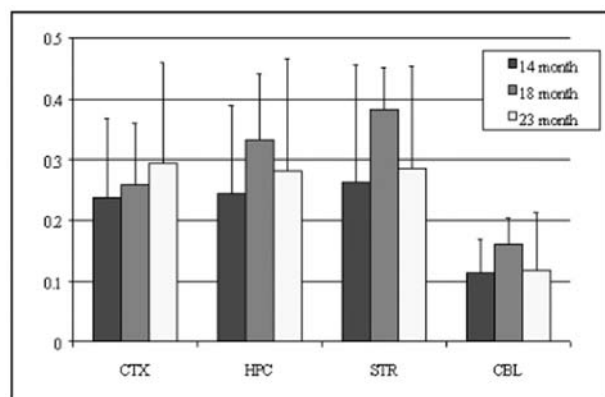


Figure 6. Soluble APPs β we levels in cortex (CTX), hippocampus (HPC), striatum (STR), and cerebellum (CBL) of aging Tg2576 mouse brain. Data shown are mean \pm SD of absorbance, $n = 3$.

brain.³⁷ A similar conclusion was suggested in a different hAPP tg mouse (hAPP V7171) by finding homeostatic APP catabolite levels except A β peptides in aging brain (3 to 15 months).⁶² We cannot exclude, however, the possibility that equivalent changes in both APPs β we generation and clearance result in static, and possibly regulated, levels with aging.

Cholinergic receptor agonists are among many pharmacological agents that regulate APP metabolism and down-regulate A β generation *in vitro* and *in vivo*.^{63–65} Thus, the putative toxicity of amyloid on human cholinergic neurons in the basal forebrain may: 1) enhance A β generation and amyloidogenesis in projection fields; 2) promote a vicious cycle of cholinergic loss and amyloidogenesis in AD brain; and thus 3) resurrect, in part, the cholinergic hypothesis of AD. Interestingly, despite similar levels of soluble A β peptides in tg mouse hippocampus and striatum and the highest ratio of A β 42/A β 40 found in the striatum, amyloid plaques were more abundant in the hippocampus than striatum—a brain region with dense cholinergic innervation. Again invoking a vicious cycle, A β peptides inhibit K⁺-evoked acetylcholine release from slice preparations of rat hippocampus and cortex but not striatum.⁶⁶

In the frontal cortex of 8-month-old Tg2576/hPresenilin-1 M146L double-tg mice immunohistological analysis reveals altered cholinergic boutons/terminals in the vicinity of amyloid plaques.²¹ Thus, amyloid plaques may cause microscopic but not macroscopic morbidity of neuronal processes and terminals leading to cholinergic deficits and synaptic loss. The relationships between clinical dementia, AD pathology, and synaptic protein levels were recently re-examined in clinically staged human AD brains obtained at autopsy. The loss of synaptic proteins is found only after the emergence of moderate to severe dementia clinically and the full spectrum of amyloid and tau pathology in the neocortex (Braak stages 5 and 6) but is not found in the presence of amyloid pathology alone. Minimal and mild clinical stages of AD are associated with either unchanged or increased levels of synaptic proteins in the neocortex, suggesting regenerative sprouting.⁶⁷ An increased density of AChE-positive fibers in the CA1 region and dentate gyrus of 17- to

22-month-old hAPP V642I (London mutation) tg mice also suggests compensatory sprouting.²⁰ In fact, amyloid-induced axonal sprouting is found in a different hAPPsw tg mouse line (not Tg2576).⁶⁸ This may explain the increased VAcHT immunoreactivity seen in Tg2576 mice at 8 months of age²¹ and the slight (but not significant) increase in ChAT activity found in striatum of tg but not wt mice at 14 months of age (Figure 3).

Normal aging does not affect cholinergic systems in mouse and rat brain.^{69–71} ChAT activity is maximal at 12 months of age in the telencephalon, brainstem, and cerebellum but there are no significant differences in telencephalic ChAT activity in mice from 6 to 30 months of age.⁶⁹ Similarly, we found that aging (from 14 to 23 months) does not affect CNS cholinergic markers in wt C57B6/SJL mice. This agrees with recent data that C57B6 mice do not exhibit age-related deficits in spatial learning or hippocampal structure.⁷² In contrast, in autopsied human brains ChAT activity in the hippocampus is maximal in the fourth decade and declines during normal aging.⁷³

We recognize that because the wt group was mostly male and the tg group mostly female, a difference between groups may have resulted from gender alone. However, we found no difference in cholinergic markers between the two groups of mice. The limited data on gender-specific cholinergic markers in aging rats suggests that alterations either require ovariectomy⁷⁴ or are slower to develop in females.⁷⁵ Finally, because female Tg2576 mice develop significantly (three times) more CNS amyloid plaque compared to males,³⁹ we may less likely detect a hypothesized amyloid-induced difference in cholinergic markers in older tg males.

If cholinergic deficits occur early in human AD, then aging Tg2576 mice fail to reproduce this aspect in addition to the lack of neuronal loss and neurofibrillary tangle pathology. However, progressive amyloidogenesis in brain in a temporal and spatial pattern similar to human AD as well as many other pathological features are recapitulated in older Tg2576 mice.^{22–40} This data favors the consensus that amyloidogenesis is necessary but not sufficient to cause AD. The mediators of cholinergic denervation in human AD brain may be neuronal loss and neurofibrillary tangle formation but not A β or amyloid. Thus, it would be of interest to examine cholinergic markers in hAPP/h τ double-tg mice that exhibit both amyloid plaques and neurofibrillary tangles in aging brain.⁷⁶ If cholinergic deficits do not occur until late stages of human AD, then older Tg2576 mice may best resemble preclinical and early stages. Thus, Tg2576 mice may be useful for testing hypotheses and therapeutic strategies based on progressive CNS amyloidogenesis but not on cholinergic deficits.

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